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THE EFFECT OF PHYSICAL CONDITIONING ON THE METABOLISM OF LACTATE, PHOSPHATE AND GLUCOSE IN RAINBOW TROUT, *SALMO GAIRDNERI*

by

Brian Ralph Hammond

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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DEPARTMENT OF ZOOLOGY

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FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Effect of Physical Conditioning on the Metabolism of Lactate, Phosphate and Glucose in Rainbow Trout, Salmo gairdneri" submitted by Brian Ralph Hammond in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Physiological effects of physical conditioning to water current were studied on three groups of 2½-year-old rainbow trout, Salmo gairdneri, acclimated to 4 C. Group One (control) were raised in still water. Groups Two and Three were conditioned to water velocities of 20 cm/sec and 40 cm/sec respectively for 16 days before sampling. Muscle and plasma samples were collected before exercise and 4 times during subjection to 15 minutes of forced swimming at 53.4 cm/sec and 8 times during a 24 hour recovery period. Conditioning significantly delayed the point of fatigue during forced exercise: the unconditioned fish were fatigued after about 5 minutes swimming, Group Two after about 10 minutes swimming and Group Three at about 15 minutes.

Analysis of muscle tissue and plasma lactate showed that conditioned fish are capable of greater production and more rapid removal of lactate from the skeletal muscle. Analysis of muscle tissue and plasma phosphate showed that exercise resulted in parallel oscillating concentration fluctuations of tissue phosphate and significant increases in concentrations of plasma phosphate in all groups. There was no significant difference between the groups of fish. Exercise resulted in increased levels of plasma glucose during recovery, although due to variability no significant difference between groups was shown.

The increase in endurance to strenuous activity provided by physical conditioning is associated with more efficient utilization of anaerobic glycolysis.

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INTRODUCTION

Fisheries management is based on scientifically proven facts incorporated into long term programs. The use of fish hatcheries is well established in these programs although proven to be expensive in stream management. Miller (1953) found that hatchery-reared trout suffered far greater mortality than wild trout when both were planted in suitable streams. He therefore initiated a research program aimed toward producing a hatchery-reared trout of superior physiological condition. His first attempts were directed towards raising a hatchery trout with increased glycogen reserves (Miller 1959). For this study he concentrated on the differences produced by various diets. Miller later designed research, reported by Hochachka (1961), to assess the effect of physical conditioning on glycogen utilization during strenuous exercise. Hochachka (1961) found that physically conditioned trout were able to utilize more of their glycogen reserves. He therefore assumed that the physically conditioned trout produced more lactate and indeed suggested that lactate might be a limiting factor in endurance.

As a contribution to this program envisioned by Dr. Miller this thesis research was planned to study the physiological effects of physical conditioning of rainbow trout to continuous water current. In particular we wished to study the pattern of lactic acid concentration changes during exercise of conditioned and unconditioned fish, since the response of this important metabolite under these conditions was unknown. Plasma glucose and plasma and tissue phosphate were measured with the assumption that these should also serve as indicators of carbohydrate metabolism changes following physical conditioning.

The rationale for using these three compounds in a physiological study is well established in the biochemical literature. Black et al.(1961) have shown that the amount of lactic acid produced by strenuously exercised fish is in direct relation to the amount of work done. They also showed that the level of blood glucose increased during activity and remained high until the 12th hour of rest. Nakatoni (1956) found significant increases in the inorganic phosphate of muscle tissue after strenuous swimming. Under severe exercise fish are unable to meet energy requirements by aerobic metabolism. As a result they are forced into anaerobic metabolism (glycolysis) and lactic acid is the end product. As shown by Black (1960) the increase of lactate in the muscle is very rapid during activity and decreases only after swimming has stopped. Black (1960) has also found that the blood lactate level continues to rise for a period of two hours after strenuous swimming and approaches the resting level after 12 hours recovery.

Because glucose is an intermediary compound between stored energy and the active muscle, it should also be an indicator of physical condition. Glucose is produced from two main sources. The conversion of glycogen to glucose by the liver is the immediate source. Under prolonged stress, proteins and fats are converted to glucose. The glucose made available is transported by the blood and enters the active cells where it supplies energy for glycolysis.

Inorganic phosphate is produced when high energy phosphate bonds in the form of ATP (adenosine triphosphate) are used for muscle contraction. The amount of inorganic phosphate produced should indicate the amount of work done. If all fish performed equal work, any changes in inorganic phosphate should reflect the effect of conditioning.

METHODS

Maintenance of Experimental Fish.

The experiments reported were carried out during the summer of 1963 at the Provincial Fish Hatchery in Calgary, Alberta. Rainbow trout, Salmo gairdneri, were hatched in November 1961, transported to outdoor rearing ponds in June 1962, and returned to the hatchery in the spring of 1963. The fish were held in three identical circular tanks. Each tank was 3.94 m in diameter, with the bottom sloping toward the center. The water measured 21 cm deep at the edge of the tank and 61 cm deep at the center. Water was squirted into the tank under pressure from five jets mounted on a water pipe positioned horizontally over the water surface. Waste water was removed by way of a standpipe in the center of the tank. All groups were fed Clark's "New Age" pellet diet at equal rates. The fish were not fed the day samples were taken. The water was saturated with oxygen and maintained at a temperature of 4 C.

Current acclimation was begun May 16, 1963. A constant rotating water current was created in two of the three circular tanks by adjusting the angle of inflowing jets of water. Two groups of trout, hereafter referred to as Group Two and Group Three, were subjected to gradually increasing water current over an eight day period. The final surface current velocity (determined by floating chip method) for Group Two was 20 cm/sec and for Group Three 40 cm/sec, a velocity determined empirically as requiring close to the maximum sustained effort by the conditioned trout. With gradual conditioning over eight days, all trout were able to maintain position in the current indefinitely without increased mortality. A control group, Group One, was

held in low current and showed no current orientation.

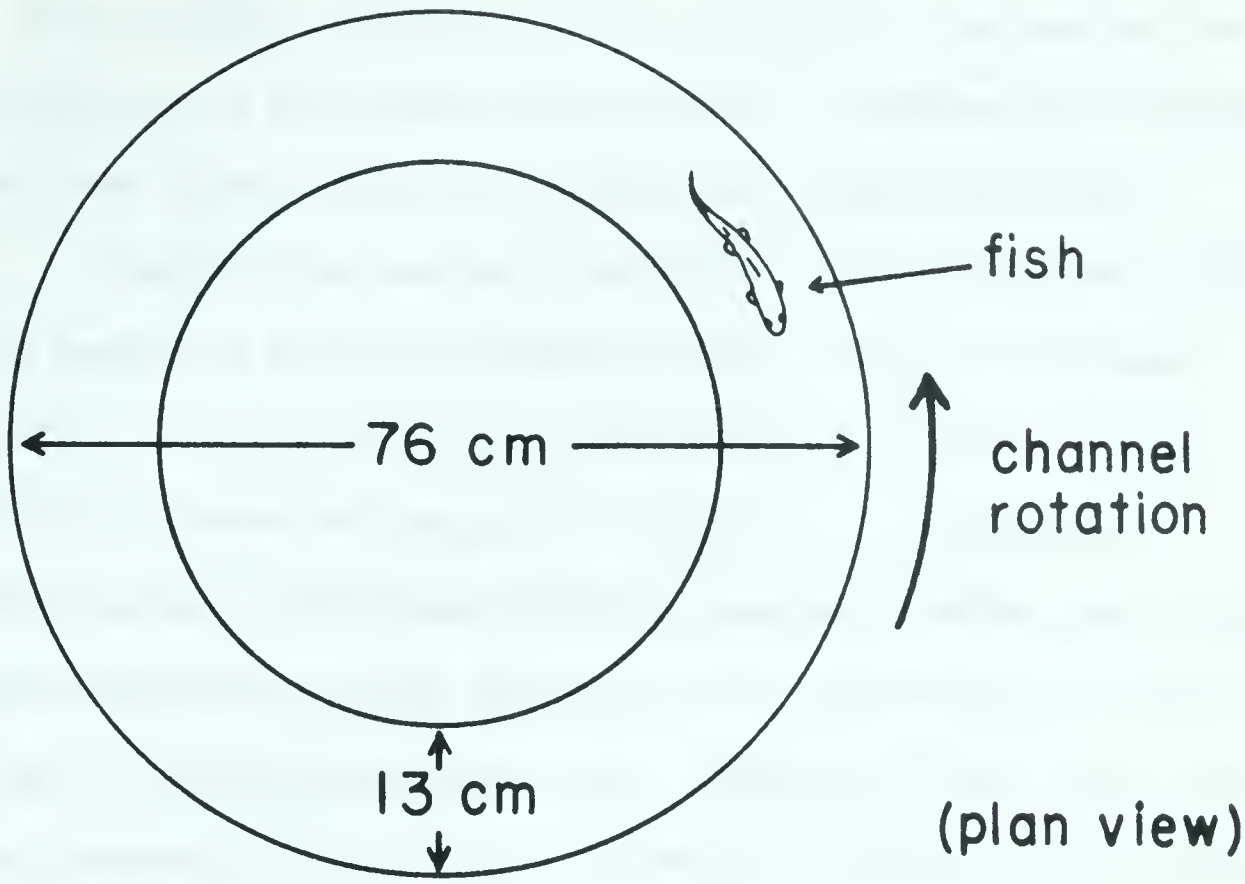
Exercise and Sampling Procedure.

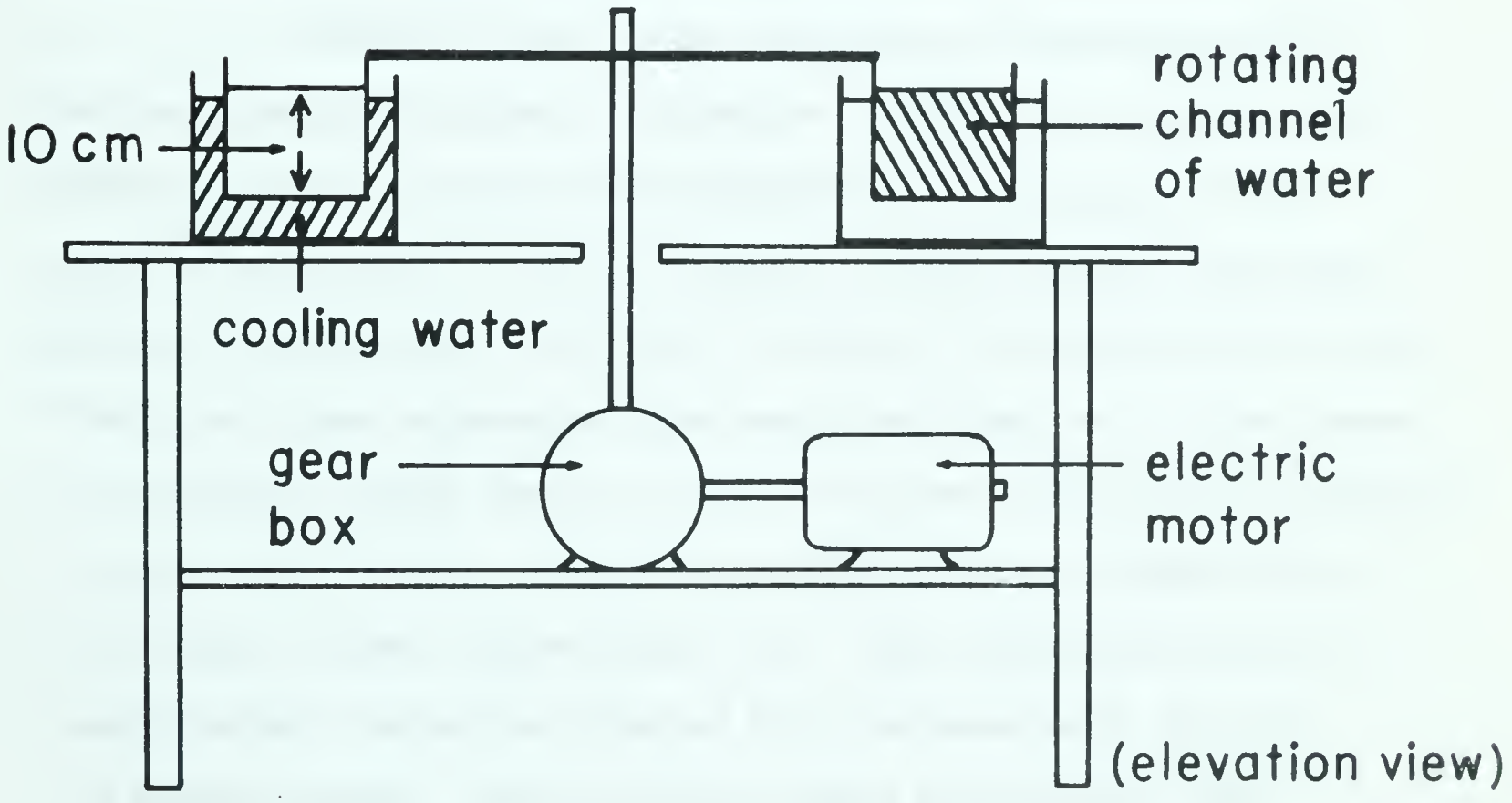
On June 8, 1963, sampling was begun, 16 days after all fish had been established in their final current velocities. The rotating fish tank (Fig. 1), used to exercise fish, was positioned adjacent to the circular conditioning tanks to facilitate rapid transfer of fish into the rotating channel of water. After being dropped into the rotating water from the handling net, all fish endeavored to maintain a constant position with respect to the operator. Trout were exercised individually at 53.4 cm/sec in the channel center. Following exercise, the fish was transferred in a pail of water to a separate covered trough (8.85 m long x 32 cm wide x 16.4 cm deep) and left undisturbed in darkness. Fish were sampled before exercise and at 2, 5, 10 and 15 minutes during the 15 minute exercise period and at 0.25, 0.5, 1, 2, 4, 8, 16 and 24 hours following exercise (recovery period). A 400 µl blood sample was taken from the ventral aorta with a heparinized tuberculin syringe while holding the fish under water. The plasma was immediately separated in a microcentrifuge. The fish was killed by spinal transection and a skinless muscle sample weighing approximately 0.1 g was removed from the epaxial region anterior to the dorsal fin. This was immediately homogenized with 10 cc of 10% trichloroacetic acid in a Serval Homogenizer, and the homogenate stored frozen for subsequent lactate and phosphate analysis.

Analytical Procedure.

Glucose was analysed by the glucostat method of Keston and Telles (1956), using the Beckman-Spinco 150 Ultramicro-Analytical

Figure 1. Rotary fish tank for strenuously exercising fish at a constant speed.





Rotary Fish Tank

(Figure 1)

System. The procedure followed was that outlined in the instruction manual supplied with the Beckman-Spinco system. Samples were analysed within one hour after collection to avoid error from glycolysis.

Analysis for inorganic phosphate in the plasma and tissue was by the method of Fiske and Subbarow (1925), using the Beckman-Spinco system. For plasma analysis the procedure outlined in the Beckman-Spinco instruction manual was followed. A modification of this method was used for tissue phosphate analysis. After centrifuging the tissue homogenate, a 20 μl fraction of the supernatant was diluted with 80 μl of 10% trichloroacetic acid. Dilution of the tissue homogenate was necessary so that sample absorbance readings would be within range of bracketing standards. The analysis was then completed according to the Beckman-Spinco instruction manual.

Lactate in the plasma and tissue was determined by the micro-method of Scholander and Bradstreet (1962). The solutions used were made as directed and stored refrigerated. The ceric sulfate solution was prepared fresh for each run. Plasma proteins were precipitated by adding 20 μl of plasma to 150 μl of 10% trichloroacetic acid. Plasma and tissue supernatant (previously described) were stored frozen. For analysis a 100 μl sample of plasma or tissue supernatant was pipetted into the ring well of a porcelain microdiffusion dish (Thomas 4472-J) fitted with a rubber sealed plastic lid. Five hundred microliters of semicarbazide solution was pipetted into the center well. With the lid slightly opened, 100 μl of ceric sulfate solution was added to the ring well and the lid immediately sealed in a clamping rack. The rack was gently rotated and the mixed samples were then incubated at 37C for 2 to 3 hours. Plasma and tissue samples were treated alike. The reagent blank was prepared by substituting water in the ring well

for the plasma or tissue supernatant. Standards containing 100 mg lactate/100 ml were prepared in the same way. For the readings, 250 µl of the semicarbazidealdehyde complex from the center well was added to 2 ml of distilled water in the cuvette of a Hitachi Perkins-Elmer 139 spectrophotometer. Absorbance was read at 223 mµ against the reagent blank set at zero. Plasma lactate was calculated as:

$$\text{mg lactate/100 ml} = 100 \times \frac{\text{unknown absorbance}}{\text{known absorbance}}$$

Tissue lactate was calculated as:

$$\text{mg lactate/g tissue} = \frac{100 \times \frac{\text{unknown absorbance}}{\text{known absorbance}}}{10 \times \text{tissue weight.}}$$

Statistical Analysis.

A one-way analysis of variance program was prepared with the aid of Dr. K. W. Smillie of the Department of Computing Science. The data was punched on cards using a Fortran Format and processed with the prepared program at the University Computing Center. The means and 95% confidence interval for each variable at each sampling time were plotted (Fig. 2 - 12).

RESULTS

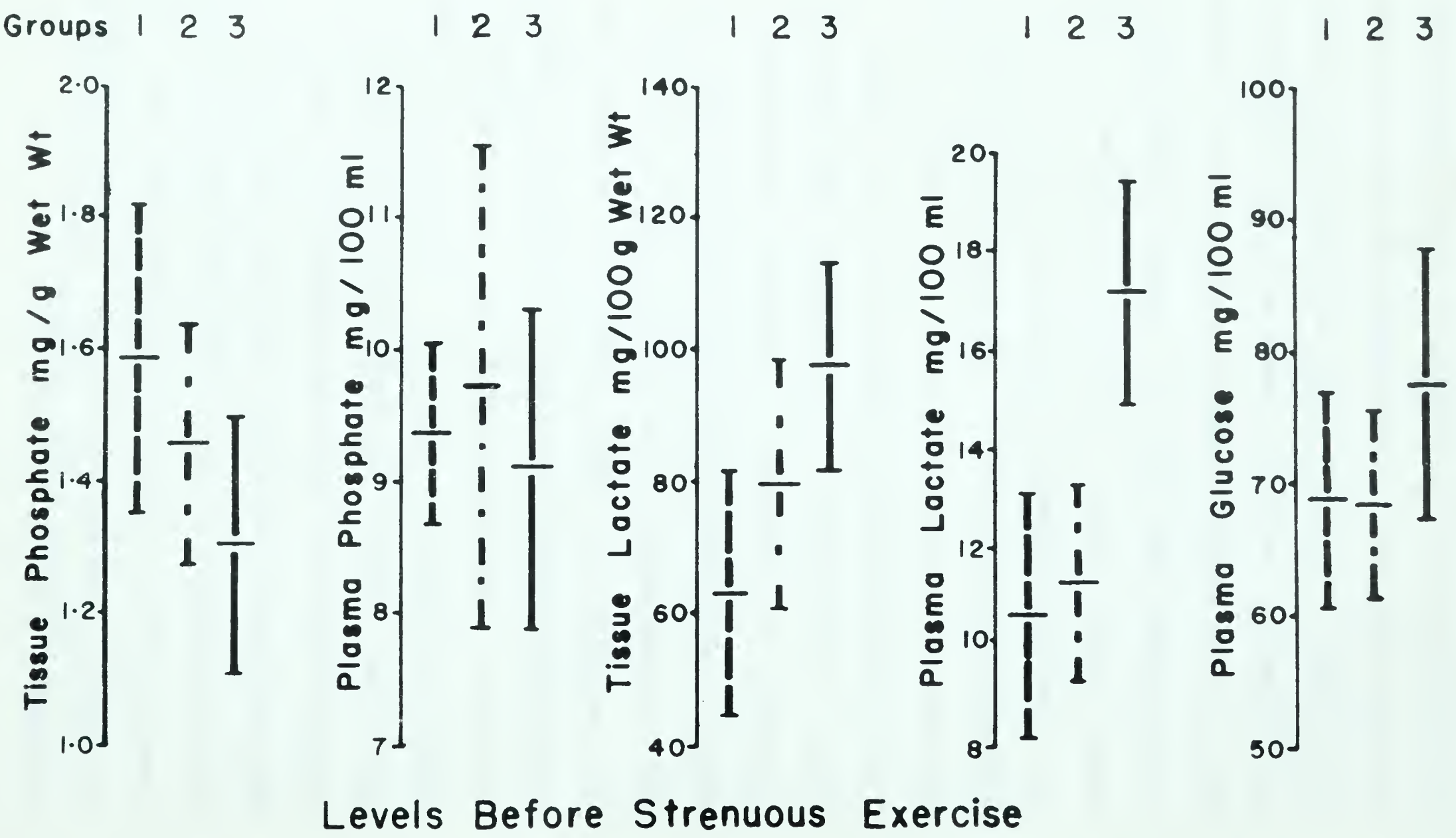
Effect of physical conditioning on resting levels of metabolism.

The resting levels of plasma and muscle lactate and phosphate and plasma glucose in the three groups of trout are shown in Fig. 2. The plasma lactate level of Group Three trout (17.0 mg/100 ml) is significantly greater at the 95% confidence level than lactate of Group One (10.6 mg/100 ml) and Group Two (11.3 mg/100 ml). Muscle lactate is also increased by conditioning with the level of Group Three trout (96.8 mg/100 g) being significantly greater at the 90% confidence level then the lactate of Group One (63.5 mg/100 g). In contrast, plasma and muscle inorganic (free) phosphate levels appear to be decreased by conditioning although again these are procliv-ities rather than statistically significant differences. In general the effect of conditioning on the resting levels of lactate, phosphate and glucose is small.

Effect of physical conditioning on endurance during strenuous activity.

The three groups of fish showed marked differences in resistance to fatigue during forced exercise in the rotating channel of water. It was observed that the Group One controls fatigued after approximately 5 minutes and were no longer able to maintain position in the current without mechanical stimulus (nudging from behind with a metal probe). Group Two fish fatigued after approximately 10 minutes. Group Three fish showed the most stamina and fatigued at approximately 15 minutes of exercise. These differences in endurance were marked and always observed during the exercise period but were not critically timed.

Figure 2. Pre-exercise concentrations of the metabolites analysed: tissue phosphate, plasma phosphate, tissue lactate, plasma lactate, and plasma glucose. Sample size was 20 fish for each group. The 95% confidence interval is shown.


Groups 1 2 3
Tissue Phosphate mg/g Wet Wt
2·0
1·8
1·6
1·4
1·2
1·0
Plasma Phosphate mg/100 ml
12
11
10
9
8
7
Tissue Lactate mg/100 g Wet Wt
140
120
100
80
60
40
Plasma Lactate mg/100 ml
20
18
16
14
12
10
8
Plasma Glucose mg/100 ml
100
90
80
70
60
50
Levels Before Strenuous Exercise

Response to exercise: tissue phosphate.

The effect of exercise on the levels of tissue phosphate is characterized by large fluctuations in all three groups (Fig. 3 and 4). Particularly interesting are the striking parallelisms between the three groups and the wave-like oscillating form of the fluctuations. Within any given sampling period, the differences between group means are not statistically significant. However, the changes between times within a group are frequently of statistical significance. In Group One the phosphate concentrations at 5 minutes (1.79 mg/g wet wt) and 15 minutes (1.72 mg/g) are significantly greater than those at 2 minutes (1.32 mg/g) and 10 minutes (1.29 mg/g)(Fig. 3). Similar significance is shown by Group Two: phosphate concentrations at 5 minutes (1.66 mg/g wet wt) and 15 minute (1.56 mg/g) are significantly greater than those at 2 minutes (1.08 mg/g) and 10 minutes (1.05 mg/g)(Fig. 3). Phosphate concentrations were more variable in Group Three and the changes in mean concentrations are as a result not statistically significant from one another. The pattern of change in Group Three is nevertheless essentially the same as that of Groups One and Two (Fig. 3). Following the cessation of forced exercise, free tissue phosphate concentrations of all three trout groups showed a similar pattern of stabilization followed by a gradual decline to pre-exercise levels (Fig. 4). Phosphate concentrations in Groups Two and Three rose somewhat during the first 15 minutes of recovery, then fell gradually. The lowest concentrations were measured after 8 hours of rest (Fig. 4). After 24 hours of recovery the levels were not significantly different from the resting levels and the tissue phosphate concentrations of all three groups are nearly identical (Fig. 4).

In summary, exercise produces large oscillations in tissue

Figure 3. Tissue phosphate concentrations (mg/g wet wt) during 15 minutes of strenuous exercise. Sample size for each group: 20 fish for initial sampling time (pre-exercise), and 10 fish for each sampling time during exercise and recovery period. The 95% confidence interval is shown.

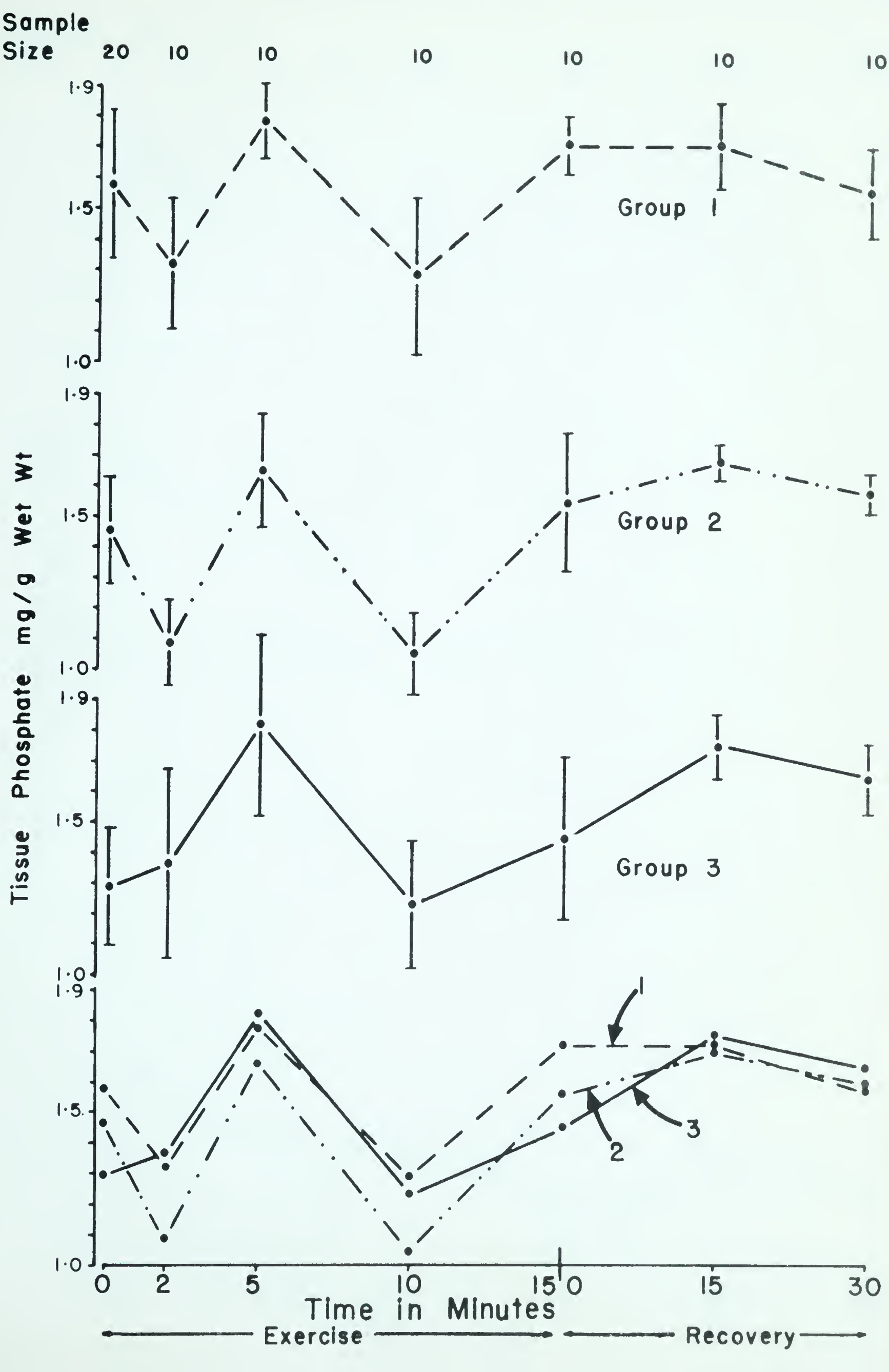

Sample Size
20
10
10
10
10
10
10
Group 1
Group 2
Group 3
Tissue Phosphate mg/g Wet Wt
1·9
1·5
1·0
1
2
3
0
2
5
10
15
0
15
30
Time in Minutes
Exercise
Recovery

Figure 4. Tissue phosphate concentrations (mg/g wet wt) during 24 hours of recovery. Sample size for each group was 10 fish for each sampling time. The 95% confidence interval is shown.

Sample Size 10 10 10 10 10 10 10

Group 1

Group 2

Group 3

Tissue Phosphate mg / g Wet Wt

1·9
1·5
1·0

1
2
3

0 1 2 4 8 16 24

Time in Hours

Recovery Period

phosphate concentrations which stabilize during recovery at levels somewhat above resting. The decline to resting levels is gradual over a period of several hours. No significant difference in tissue phosphate between the three group means was observed.

Response to exercise: plasma phosphate.

Exercise caused a rapid and significant increase in the plasma phosphate levels of all three groups of trout (Fig. 5 and 6). All three groups showed the same general pattern of response: plasma phosphate increased sharply during the first two minutes (average rise 27.8%), then declined slightly at 5 minutes. Between 5 and 10 minutes, the plasma phosphate concentrations again increase (average increase 40.0%) to reach peak or near-peak levels. Further exercise (between 10 and 15 minutes) has no significant effect on plasma phosphate concentrations (Fig. 5). Following exercise, plasma phosphate falls gradually and for Groups One and Three the 24 hour recovery levels are significantly higher than the original resting levels (Fig. 6).

In summary, conditioning to water current makes no significant difference in the plasma phosphate levels between groups at any one sampling time.

Response to exercise: tissue lactate.

Figures 7 and 8 show the effect of strenuous exercise on the levels of tissue lactate. All groups follow a similar tissue lactate response pattern. The most rapid increase occurs during the first two minutes of exercise (average increase 180.5%). Control Group One tissue lactate reaches a peak (264.03 mg/100 g wet wt) after 5 minutes then falls during the remaining 10 minutes of exercise. Group Two reaches a peak

Figure 5. Plasma phosphate concentrations (mg/100 ml) during 15 minutes of strenuous exercise. Sample size for each group: 20 fish for initial sampling time (pre-exercise), and 10 fish for each sampling time during exercise and recovery period. The 95% confidence interval is shown.

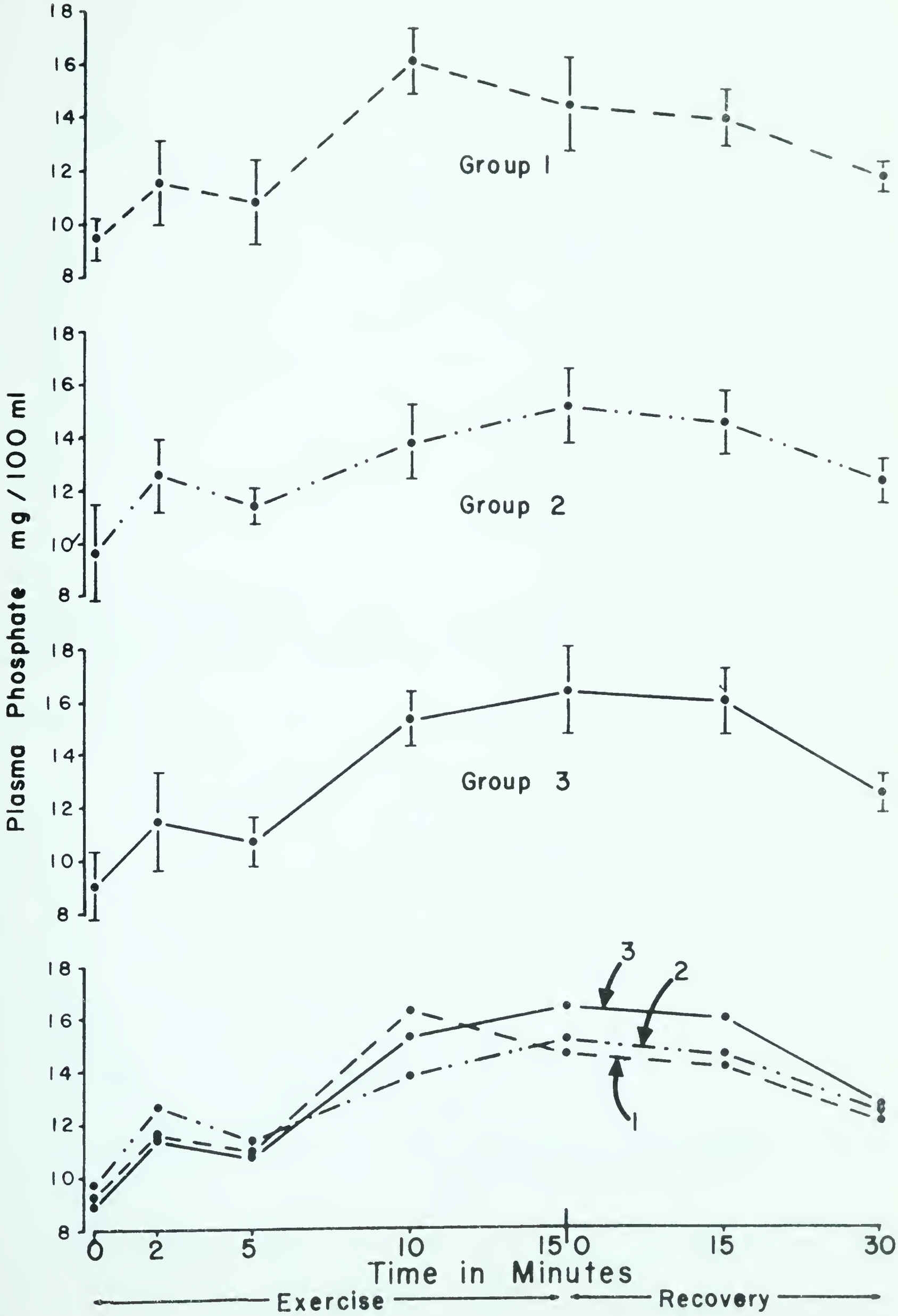
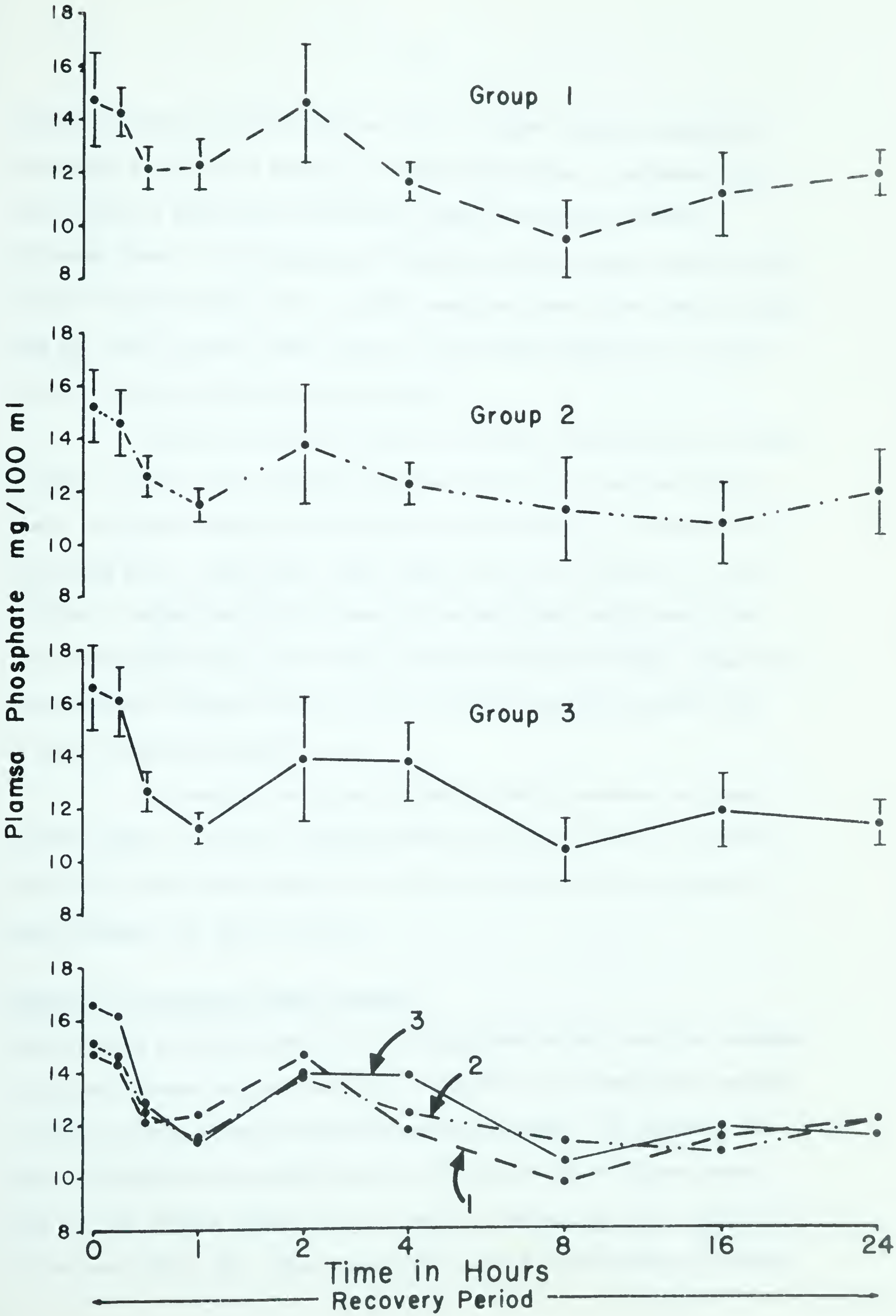

Plasma Phosphate mg / 100 ml
18
16
14
12
10
8
Group 1
Group 2
Group 3
3
2
1
0
2
5
10
15
0
15
30
Time in Minutes
Exercise
Recovery

Figure 6. Plasma phosphate concentrations (mg/100 ml) during 24 hours of recovery. Sample size for each group was 10 fish for each sampling time. The 95% confidence interval is shown.


Plamsa Phosphate mg/100 ml
18
16
14
12
10
8
Group 1
Group 2
Group 3
3
2
1
0
1
2
4
8
16
24
Time in Hours
Recovery Period

(290.32 mg/100 g) after 10 minutes with a slight decrease during the remaining 5 minutes of exercise. Group Three shows a continual rise and reaches a peak (322.18 mg/100 g) after 15 minutes exercise. Although there is no significant difference between peak concentrations of the 3 groups,only at the 5 minute sampling time is the control Group One not significantly lower than the conditioned Groups Two and Three in the 15 minute exercise period (Fig. 7).

During the first 8 hours of recovery the decrease in tissue lactate is very rapid (average decrease 65.0%). For the remaining 16 hours no further significant decrease is shown (Fig. 8). Physical conditioning has a significant effect upon the rate of removal of tissue lactate. During the first 8 hours of recovery the conditioned Group Three shows the most rapid removal (average decrease 71.0%), Group Two intermediate (average decrease 64.4%) and the control Group One the slowest (average 58.5%)(Fig. 8).

In summary, exercise produces a large increase in tissue lactate which is removed rapidly during the first 8 hours of recovery. Physical conditioning extends the period of tissue lactate production and increases its rate of removal.

Response to exercise: plasma lactate.

Conditioning to water current has a significant effect upon the response of plasma lactate to exercise (Fig. 9 and 10). All three groups exhibit a similar linear increase during exercise (increase 3.08 mg/min). However the conditioned Group Three has an increase (0.54 mg/min) twice that of the control Group One (0.27 mg/min) during the first two hours of recovery (Fig. 10). The peak plasma lactate concentration of Group

Figure 7. Tissue lactate concentrations (mg/100 g wet wt) during 15 minutes of strenuous exercise. Sample size for each group: 20 fish for initial sampling time (pre-exercise), and 10 fish for each sampling time during exercise and recovery period. The 95% confidence interval is shown.

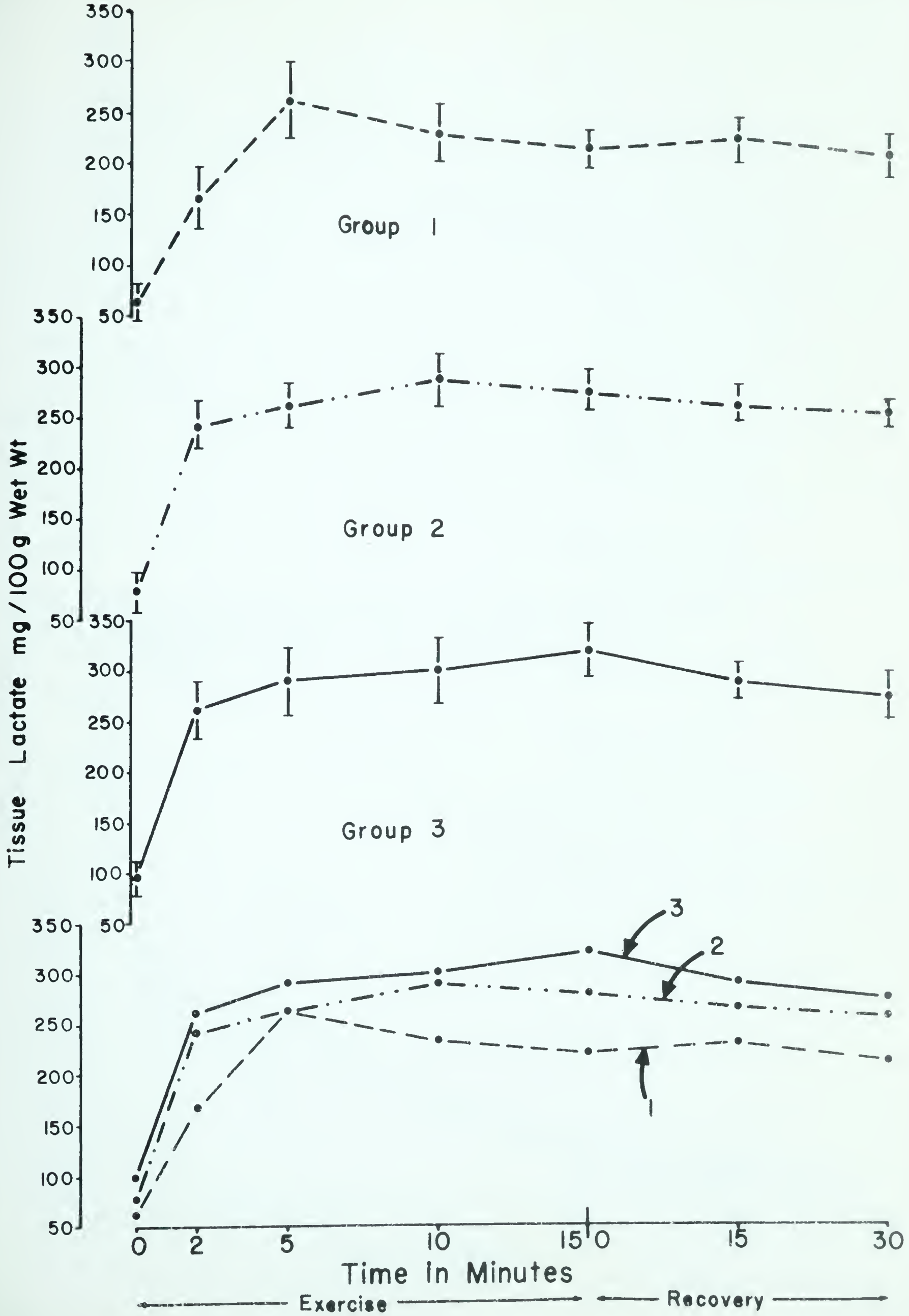
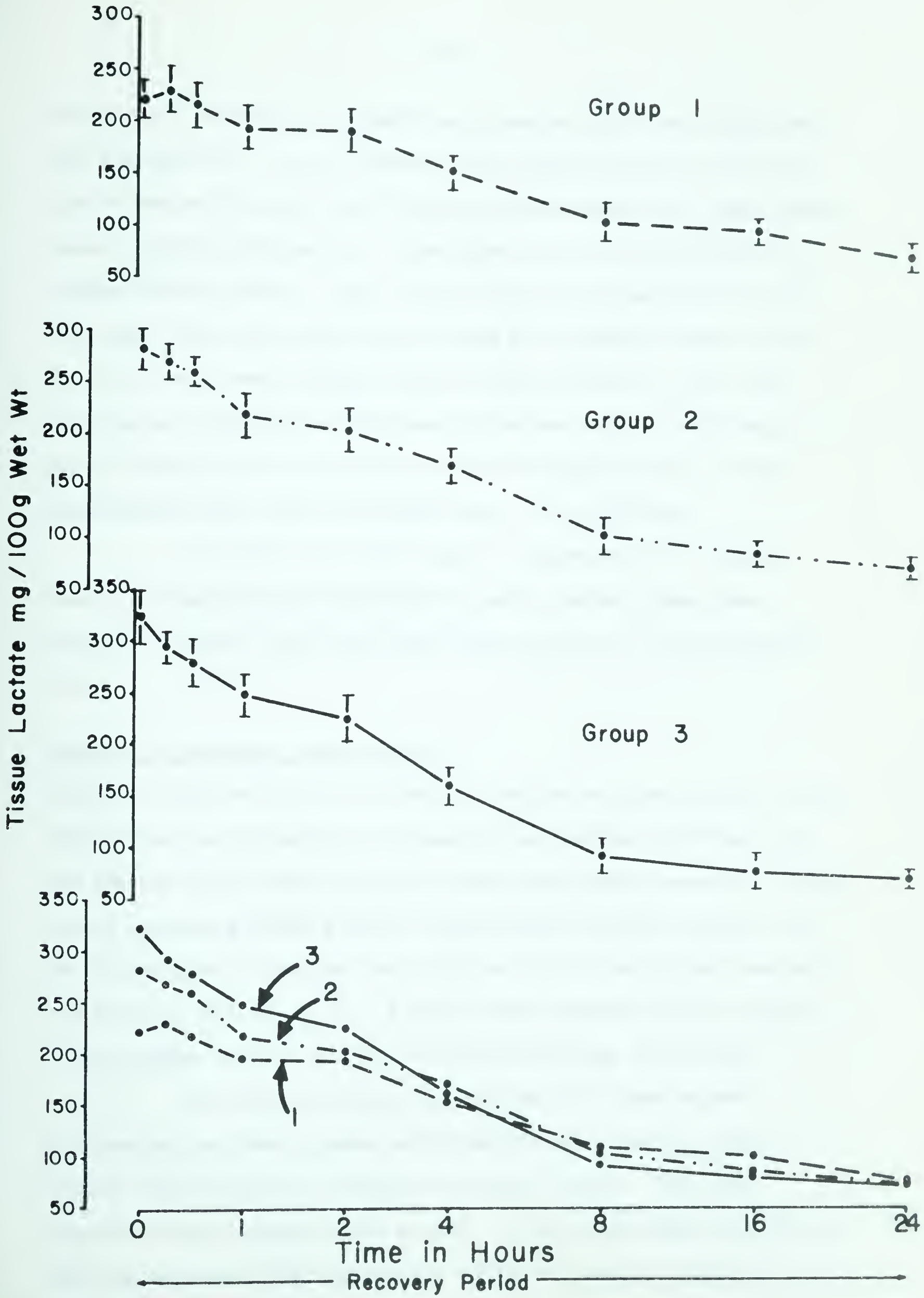

Tissue Lactate mg / 100 g Wet Wt
350
300
250
200
150
100
50
Group 1
Group 2
Group 3
3
2
1
0
2
5
10
15
0
15
30
Time In Minutes
Exercise
Recovery

Figure 8. Tissue lactate concentrations (mg/100 g wet wt) during 24 hours of recovery. Sample size for each group was 10 fish for each sampling time. The 95% confidence interval is shown.


Tissue Lactate mg / 100g Wet Wt
300
250
200
150
100
50
Group 1
Group 2
Group 3
350
3
2
1
0
1
2
4
8
16
24
Time in Hours
Recovery Period

Three (126.7 mg/100 ml) is significantly greater than that of Group One (89.9 mg/100 ml). It is of interest that plasma lactates of all three groups peak at the same time (2 hours recovery) despite the large differences in peak concentrations. Group Three has the most rapid rate of plasma lactate removal. After 8 hours of recovery Group Three is significantly lower than Group One (30.4 and 45.4 mg/100 ml respectively) (Fig. 9). This trend continues until 24 hours recovery at which time there is no statistically significant difference between group means. After 24 hours recovery only Group Three (9.6 mg/100 ml) has a level significantly lower than the resting level (17.0 mg/100 ml).

The effect of conditioning is manifested in the higher exercise-induced plasma lactate levels and in the more rapid rate of removal of lactate from circulation during recovery of the conditioned fish.

Response to exercise: plasma glucose.

Figure 11 shows the effect of strenuous exercise on plasma glucose levels. Despite the high variability of plasma glucose amongst individual fish and the resulting instability of the group means during exercise, a trend toward increasing plasma glucose concentrations following exercise can be distinguished. Group One has levels at 4,8, 16 and 24 hours recovery and Group Two has levels at 4, 8 and 24 hours recovery that are significantly higher than the original resting levels (Fig. 11 and 12).

The effect of strenuous exercise is to cause marked fluctuations in plasma glucose during and following exercise with an overall rise in glucose concentration during recovery. This post-exercise increase appears to be greater in the unconditioned fish (Group One) but because of high variability within the groups, no difference is statistically significant.

Figure 9. Plasma lactate concentrations (mg/100 ml) during 15 minutes of strenuous exercise. Sample size for each group: 20 fish for initial sampling time (pre-exercise), and 10 fish for each sampling time during exercise and recovery period. The 95% confidence interval is shown.

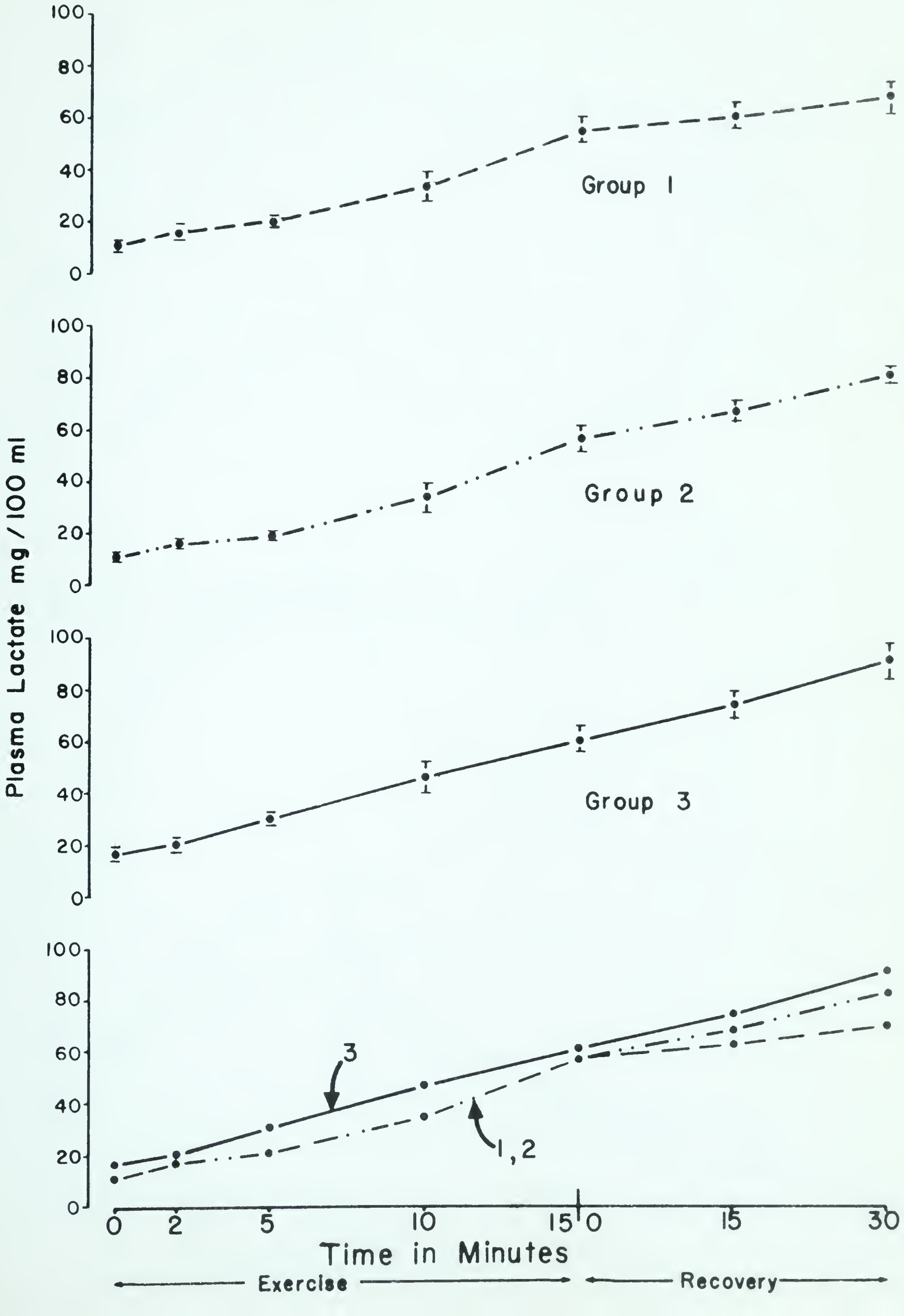

Plasma Lactate mg / 100 ml
100
80
60
40
20
0
Group 1
Group 2
Group 3
3
1, 2
0
2
5
10
15
0
15
30
Time in Minutes
Exercise
Recovery

Figure 10. Plasma lactate concentrations (mg/100 ml) during 24 hours of recovery. Sample size for each group was 10 fish for each sampling time. The 95% confidence interval is shown.

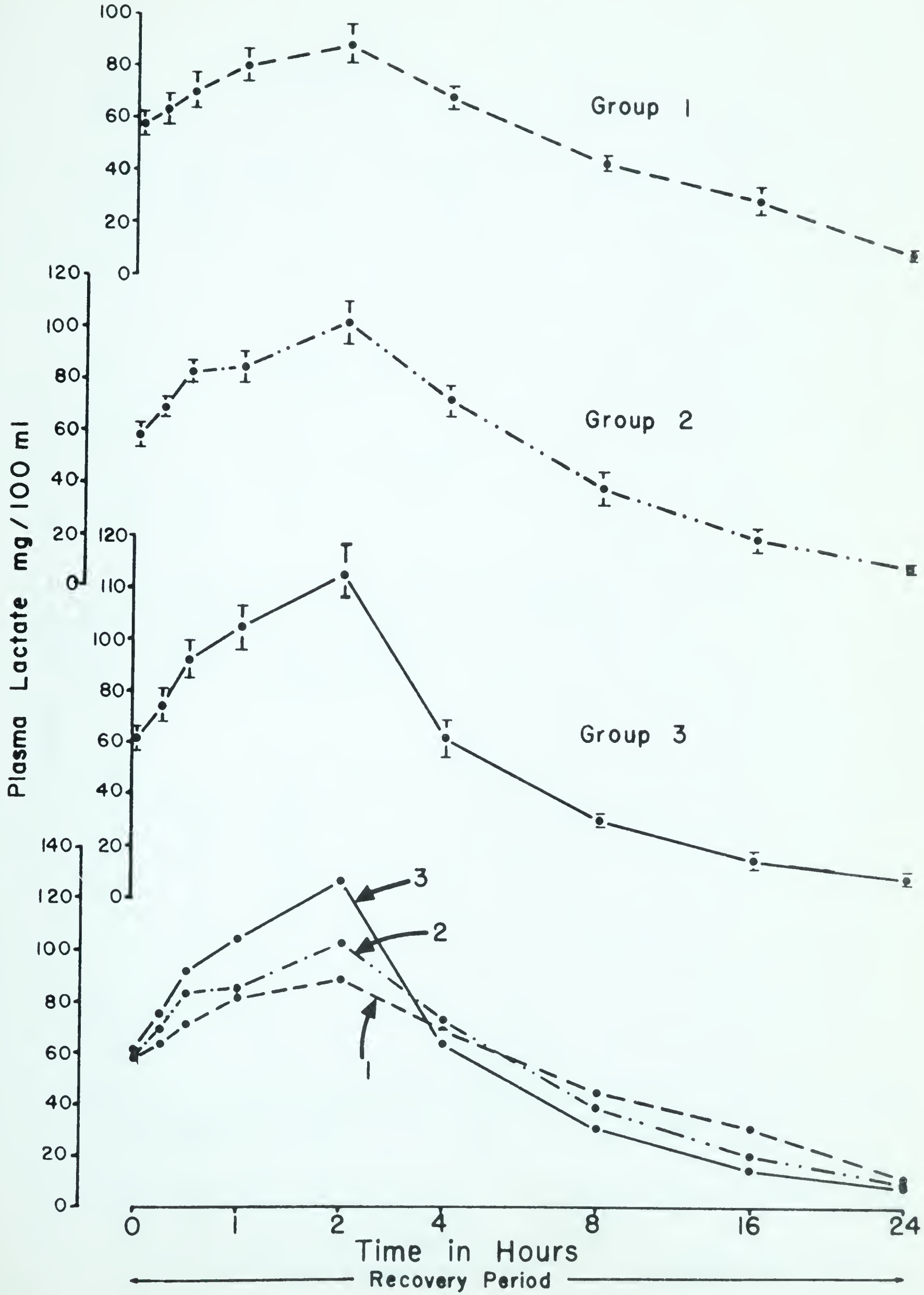

Plasma Lactate mg/100 ml
Group 1
Group 2
Group 3
1
2
3
0
1
2
4
8
16
24
Time in Hours
Recovery Period

Figure 11. Plasma glucose concentrations (mg/100 ml) during 15 minutes of strenuous exercise. Sample size for each group: 20 fish for initial sampling time (pre-exercise), and 10 fish for each sampling time during exercise and recovery period. The 95% confidence interval is shown.

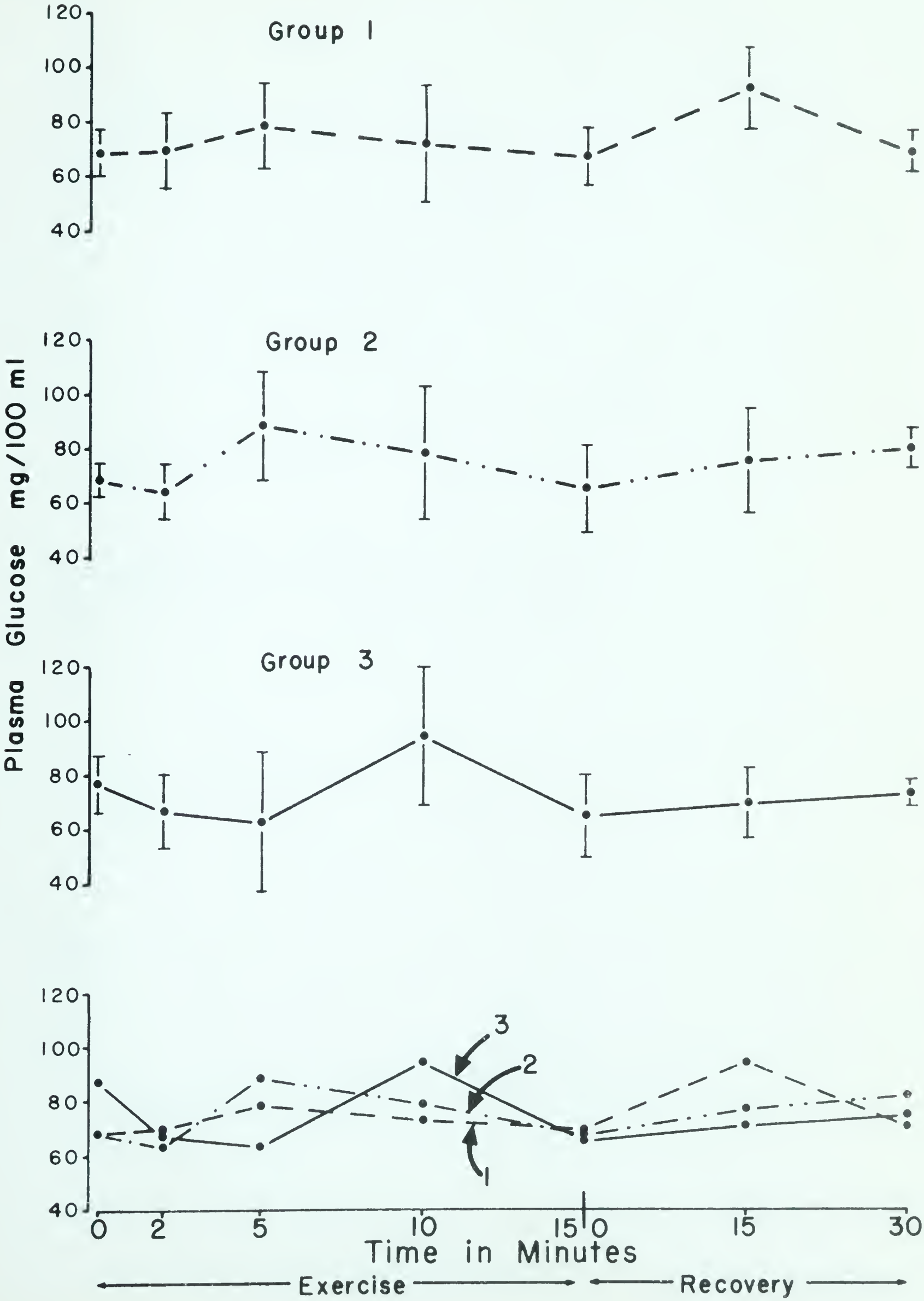
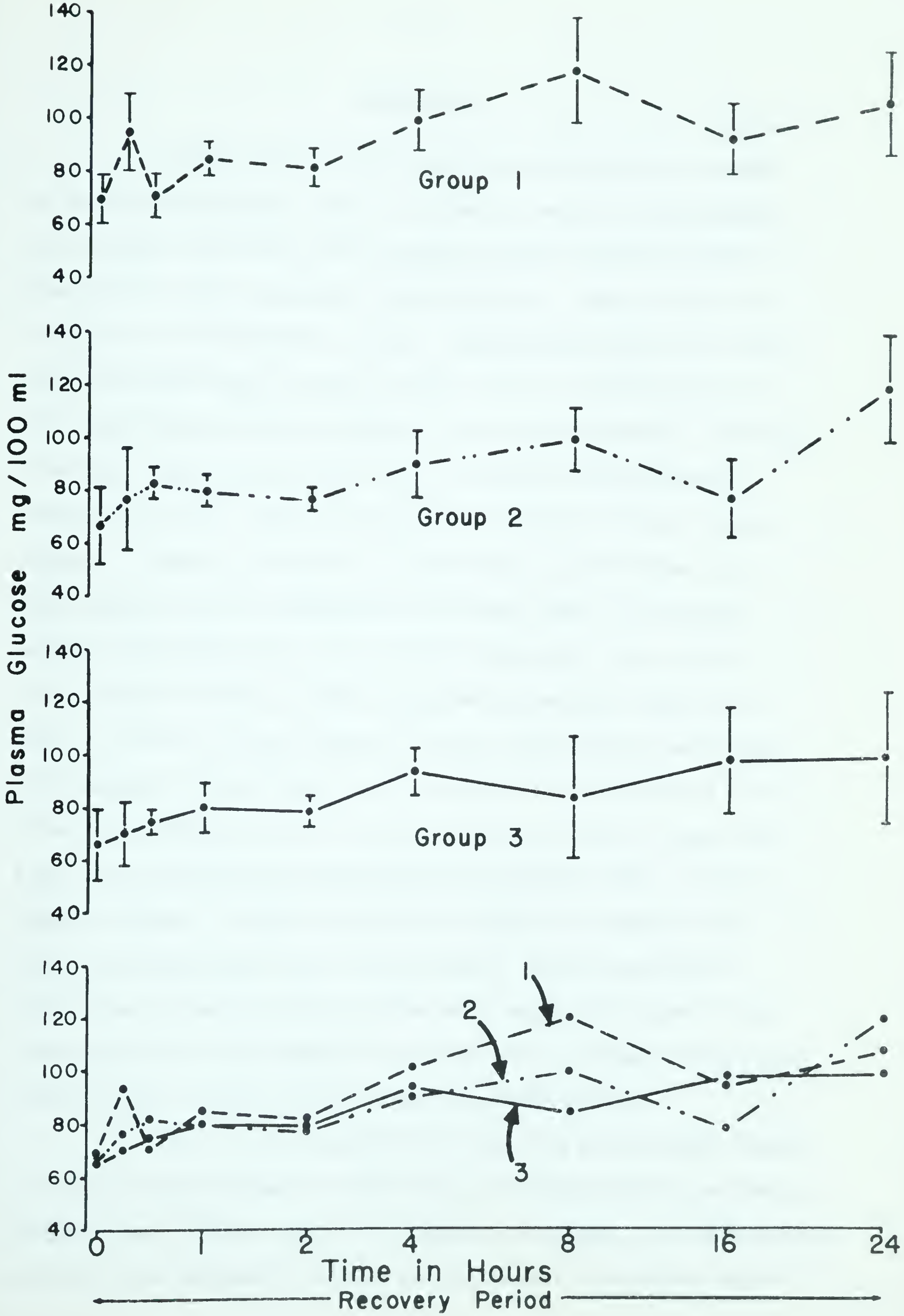

Group 1
Group 2
Group 3
120
100
80
60
40
Plasma Glucose mg/100 ml
3
2
1
0
2
5
10
15
0
15
30
Time in Minutes
Exercise
Recovery

Figure 12. Plasma glucose concentrations (mg/100 ml) during 24 hours of recovery. Sample size for each group was 10 fish for each sampling time. The 95% confidence interval is shown.


Plasma Glucose mg/100 ml
140
120
100
80
60
40
Group 1
Group 2
Group 3
1
2
3
0
1
2
4
8
16
24
Time in Hours
Recovery Period

DISCUSSION

Energy required for physical work is supplied to muscle as adenosine triphosphate (ATP). Because the amount of stored muscle ATP is small, and because ATP is rapidly consumed during exercise, a means for its rapid replacement must be present. Three pathways are recognized for the formation of ATP. Transphosphorylation, the transfer of the high-energy phosphate bonds, stored as phosphocreatine or other high energy phosphate compounds, provides an immediate reservoir of energy. Rephosphorylation of ADP to form ATP with high-energy phosphate from this reservoir is rapid and important for the initial response. However, the supply is limited and only sufficient for a short period of work (Huennekens and Whiteley 1960). The second method of ATP production is by anaerobic glycolysis. This is the major pathway yielding the most energy during exercise (White et al 1959). In trout the large amount of lactic acid produced during exercise indicates extensive use of this pathway. The third source of ATP is oxidative phosphorylation, the most effective method of generating high-energy phosphate bonds (Huennekens and Whiteley 1960). During exercise, however, energy utilization by muscle far outstrips the energy-yielding capabilities of this system. In fish especially, limitations of the respiratory system and a meager blood flow to the trunk musculature make impossible any substantial increase during exercise of overall energy yield by oxidative phosphorylation.

This research endeavored to study the physiological effects of physical conditioning on rainbow trout, especially effects on chemical pathways that provide power to the locomotive machinery. The measurements of lactate and phosphate in muscle and plasma give considerable infor-

mation about sources of energy used in muscular contraction. Changes in the concentration of inorganic phosphate indicate the rate of ATP utilization and synthesis. Nakatani (1956, 1957) and Felton (1956) showed that severe exercise increased the level of inorganic phosphate in plasma and skeletal muscle of salmonids.

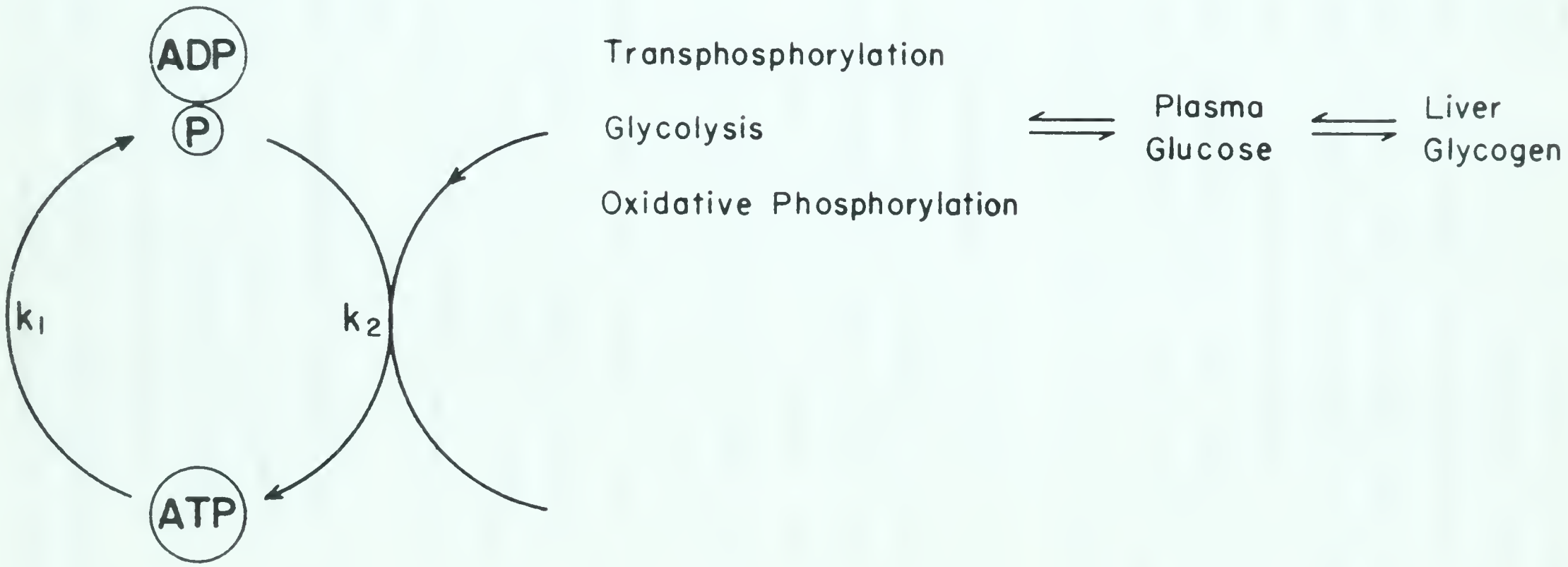
Changes in the concentration of lactic acid are indicative of the rate of glycolysis. An increase in the concentration of lactic acid is correlated with an increase in the rate of glycolysis resulting from increased work. This has previously been demonstrated by Black (1955, 1957a, 1957b, 1957c), Black et al (1959), Black et al (1960), Miller et al (1959), Miller and Miller (1962), and Nakatani (1957) working with salmonids and Secondat et al (1942) working with cyprinids. Further, increased lactic acid concentrations in plasma or muscle was found to correspond to an immediate decrease in muscle glycogen stores and a delayed decrease in liver glycogen stores with forced exercise (Black et al (1960), Black et al (1961), Miller et al (1959), and Miller and Miller (1962)

The effect of physical conditioning on the utilization of glycogen during and following strenuous exercise was studied by Hochachka (1961). He found that conditioning permitted greater utilization of stored muscle glycogen. Liver glycogen concentrations were not significantly altered during exercise but during recovery unconditioned trout showed greater depletion of liver glycogen than did physically conditioned trout. Plasma glucose is an intermediate compound between liver glycogen and glycolysis. Accordingly, changes in glucose concentration are indicative of its production and utilization. It has been shown by Black et al (1960) and Secondat (1950) that exercise increases the concentration

of plasma glucose in fish.

A cyclic model for the metabolism of inorganic phosphate is shown in Fig. 13. The explanation of this model is based on the principles used by Reimer (1959). During rest the system is in a steady state and the rate constant (k_1) for the utilization of ATP equals the rate constant (k_2) for ATP synthesis. At the start of strenuous activity the rate constant (k_1) will be increased, resulting in the rapid depletion of stored ATP and creatine phosphate. The utilization of ATP for the energy-requiring processes will automatically increase the available supply of adenosine diphosphate (ADP) and inorganic phosphate. These in turn become available to react in the coupling mechanisms and permit respiration to proceed (White et al 1960). As the anabolic processes are capable of an excess production of ATP the system over-compensates and concentrations of ADP and inorganic phosphate are greatly reduced. As a result synthesis is retarded. Not until the concentrations of ADP and inorganic phosphate increase are the anabolic processes again activated, with the result that concentrations of inorganic phosphate show significant fluctuations. Webb (1963) states that cyclic, regenerative, and feedback enzyme systems are particularly prone to overshoot and fluctuation. This model is substantiated by the significant fluctuations measured during exercise in tissue phosphate of all three groups of fish (Fig. 3). Although there is no statistically significant difference amongst group means at any sampling period, the changes in concentration during the first two minutes of activity are of interest. Groups One and Two show decreases in the tissue phosphate concentrations, while Group Three, with a lower initial concentration shows an increase in tissue phosphate. A possible explanation is based

Figure 13. Metabolic pathways for the generation and storage of energy: cyclic model of ATP metabolism and reversible pathways of plasma glucose.



Cyclic Model of ATP Metabolism

on differences in the amount of stored ATP and high-energy bonds. With the start of activity Groups One and Two are immediately forced into the production of high-energy bonds as shown by the rapid decrease in tissue phosphate. Although Group Three is also forced into glycolysis more phosphocreatine is initially available allowing the corresponding increase in tissue phosphate (Fig. 3). Group Three possibly has more phosphate stored as phosphocreatine, indicated by the lower resting level of free inorganic phosphate. Conditioned fish are therefore better prepared for emergency activity by virtue of their superior stores of high-energy phosphates.

Changes in plasma phosphate are the result of all the metabolic processes in the body and do not represent effects of muscle activity alone. Inorganic phosphate is freely diffusable and appears in circulation almost as soon as released in the cells. The increase in plasma phosphate during activity indicates that more high-energy phosphate bonds are being utilized than are being synthesized. The decrease in plasma phosphate during the first hour of recovery shows that more high-energy phosphate bonds are being synthesized. This explanation is based on work reported by Cantarow and Schepartz (1960) who found that blood phosphate decreased during the periods of increased carbohydrate utilization.

This research has demonstrated that physical conditioning greatly increases stamina in rainbow trout, an increase that is associated with certain metabolic changes in response to exercise. Of especial importance is a sustained production of muscle lactate during exercise and a more rapid removal of muscle lactate following exercise in physically conditioned trout. Because glycolysis is the major source of ATP, the concentration of lactate indicates the amount of energy

consumed. All groups presumably require the same amount of energy for an equivalent amount of work performed. As lactate is freely diffusible across the cell membrane, lactate concentration in the plasma is roughly indicative of its concentration in tissue. As expected, the conditioned Group Three has the highest plasma lactate concentration after 15 minutes of strenuous exercise. An analogous situation was reported by Robinson and Harmon (1941) who found that man's ability to accumulate lactate during anaerobic metabolism increased with physical training.

Observations of swimming stamina of the three groups of trout indicates a direct correspondence of stamina and plasma lactate concentrations. From these results it is not possible to give a definite answer to the suggestion of Hochachka (1961) that lactate acts as a limiting factor in sustained activity of trout. However, it is apparent that physically conditioned trout can continue activity in the presence of high muscle lactate levels for a longer period than can unconditioned trout.

Trout subjected to physical conditioning show compensatory reactions which lead to acclimation. Prosser (1964) suggests these changes are the result of enzyme induction or from changes in cofactors or in other modifiers of catalyzed reactions. Superior stamina and more rapid recovery following exercise of physically conditioned rainbow trout suggests that conditioning improves efficiency of at least certain steps in the metabolic system. It is known that physically conditioned and unconditioned trout mobilize nearly equal amounts of liver glycogen during the early period of recovery following exercise (Hochachka, 1961). Hence, it is expected that nearly equal amounts of plasma glucose will enter the circulation. However, if a difference in the plasma glucose is evident between the two groups, a difference in its utilization rate

is indicated. Group Three showed the least significant increase in plasma glucose during recovery, suggesting a more rapid removal of glucose from the circulation and a larger utilization in a more active metabolic system. Hochachka (1961) found physically conditioned trout capable of significant recovery of muscle glycogen after strenuous activity, while unconditioned trout showed no increase during the same period. Thus, unconditioned trout are unable to use effectively circulating glucose to resynthesize muscle glycogen. A possible explanation is found in the work of MacLeod, quoted by Black (1961), who reported a deficiency of hexokinase in muscle of steelhead trout (Salmo gairdneri). This suggests that physically conditioned trout have larger concentrations and/or a more active form of muscle hexokinase. In support of possible enzymatic differences, Hochachka and Hayes (1962) found that brook trout, (Salvelinus fontinalis), acclimated to cold water (4 C) relied mainly on the pentose cycle for their energy production, while warm (20 C) acclimated trout used predominantly the Embden-Meyerhof pathway. The fact that physically conditioned trout are capable of mobilizing larger amounts of stored energy and recovering more rapidly from fatigue, suggests that the primary effect of physical conditioning may be on enzyme systems. Further research is needed to elucidate the role of transphosphorylation as an immediate energy source, and possible differences in enzyme systems in physically conditioned fish. The possible role of lactate as a limiting factor to activity can now be studied because the activity response pattern of lactate in physically conditioned and unconditioned trout is known.

For practical benefit to fisheries management the most efficient water temperature and the minimum time required for maximum physical conditioning under various water currents must be studied.

It would be of interest to investigate the stamina response and the physiological effects of exercising physically conditioned fish at various temperatures. Such information would be of importance in fish stocking programs.

SUMMARY

1. Two and one-half year old rainbow trout, Salmo gairdneri, were acclimated at 4 C and physically conditioned to water currents of 40 cm/sec (Group Three) and 20 cm/sec (Group Two). The controls (Group One) were raised in low water current.

2. When the trout were exercised at 54.3 cm/sec, it was observed that the control Group One fatigued after approximately 5 minutes, Group Two after approximately 10 minutes and Group Three at approximately 15 minutes of forced strenuous swimming.

3. Conditioning significantly increased the pre-exercise concentration of plasma lactate. There were no statistically significant differences in the resting levels of tissue lactate, tissue phosphate, plasma phosphate and plasma glucose.

4. Plasma lactate increased during exercise, and continued to increase after exercise, reaching a peak after 2 hours of rest.

5. Physical conditioning of rainbow trout enables them to produce higher concentrations of lactate during extended periods of exercise and remove lactate more rapidly during recovery.

6. The effect of exercise on tissue phosphate was to induce significant oscillating concentration fluctuations which subsided during recovery. These fluctuations may be explained on the basis of fluctuation and overshoot described for multienzyme systems.

7. Significant increase in plasma phosphate concentrations was the result of exercise.

8. No statistically significant differences amongst groups in the concentrations of tissue and plasma phosphate were produced by physical conditioning and exercise.

9. Plasma glucose of Groups One and Two increased significantly during recovery following exercise.

10. It is suggested that the significant physiological effects of physical conditioning may be due to changes in concentration and/or activity of enzymes in the metabolic systems.

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